Structural and Regulatory Aspects of Enzymes in Chemotherapy

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Summary

A brief discussion is presented on the enzymatic changes in normal and malignant tissues after creation of metabolic stresses with Methotrexate and 6-azauridine. The possible relationship of these changes to the relative adaptive capabilities seen in normal liver and hepatomas as reported by Pitot and Potter is considered. Certain aspects of cell membrane structure and metabolism as they may relate to neoplasia and possible therapeutic approaches are presented. Finally, evidence for the subunit structure of L-asparaginase is outlined with possible means of exploitation of this finding to increase its therapeutic potential.

This paper attempts to present some speculative ideas from which experimental approaches to the therapy of cancer might arise. Given the fact that molecular and physical biology have produced many tools and conceptual advances which are already incorporated into everyday research directed toward cancer chemotherapy, what are some possible new dimensions?

The delineation of the DNA-RNA-protein dogma allows a tidy compartmentalization of the action of many antimetabolites. Unfortunately, the cancer cell, and occasionally the host, refuses to accept such a static role of these agents on their target. The metabolic stress so created can release the cell from its normal homeostasis. As a consequence, increased amounts of a target enzyme may be produced or alternative pathways may be activated. Such adaptation may be different than the selection of a few preexistent cells in the total population that are resistant to the agent by a genetic deletion or alteration in the level of an enzyme. Although the picture is still unclear, the response of mammalian cells to Methotrexate may include a derepression of the production of dihydrofollic reductase (3). The success with which individual cell types can cope with the inactivation of this enzyme may depend on the ability of the cell to increase the synthesis of the enzyme at a rate which exceeds the binding of the enzyme by free Methotrexate.

A similar case has arisen in the limited clinical trials of 6-azauridine. This agent is capable of modifying the disease state of patients with both myelogenous and lymphocytic leukemia with relatively little host toxicity. The effect, however, is short-lived despite what must be considered to be the killing of several logs of leukemic cells. Biochemical studies indicate that the initially effective action is terminated by a sharp increase in the level of the target enzyme, orotidylic acid decarboxylase (5, 7). Since 6-azauridine, after conversion to its 5'-phosphate derivative, is a competitive inhibitor of this decarboxylase, the accumulation of orotidylic acid behind the point of blockade and the increase in total enzyme activity nullify the initially effective starvation of the cell for pyrimidine nucleotides.

These two examples of circumstances where the therapeutic effects of antimetabolites may be adversely effected by "derepression" of enzyme synthesis could reflect a larger number of similar cases where therapy in man meets with limited success. We have been conditioned to seek a marked decrease of activating enzymes for purine and pyrimidine antimetabolites as a mechanism for resistance in man because of the great facility with which this type of mutant cell is selected in experimental tumor systems (11). Success in this search in patients has been very limited (6), and it might be suggested that other mechanisms such as adaptive changes in the target pathway might be appropriate for investigation.

It would seem a simple matter to deal with such compensatory increases in enzyme synthesis with the many specific inhibitors of RNA and protein synthesis that have served the cause of molecular biology with such distinction. Inherently, however, there are some flaws in the approach which must be considered. The elegant studies of Pitot and Potter (18) with minimal deviation hepatomas would suggest that in this class of tumors the malignant state can be characterized by an inability to respond to altered levels of substrate within the cell. It would appear, for example, that certain tumor lines are either firmly repressed to a very low level of serine dihydrolase while others appear to be fully derepressed regardless of the nutritional state of the animal. Under these experimental conditions, large changes in enzyme activity occur in the livers of animals bearing these tumors or control animals. A similar situation may obtain with regard to the response of normal and malignant cells to L-asparaginase. Measurement of the ability of different cell types to produce L-asparagine indicates that leukemic cells are either very deficient in this reaction, and thus sensitive to the enzyme, or they are often able to synthesize this amino acid in amounts far exceeding that found in normal cells (13, 16). Furthermore, evidence is being accumulated that normal cells can respond to L-asparaginase treatment by a marked increase in their biosynthetic ability for L-asparagine (2). It is not clear at
this time what the response of leukemic cells is to this stress, but a firm state of repression or derepression may exist.

It might be reasoned that other chemotherapeutic agents which produce stresses on a metabolic pathway would also create adjustments in normal tissues, thereby providing a measure of host tolerance to the agent. This could, in fact, favor the therapeutic response if the neoplastic cells did not have a similar capability to respond. If one now attempts to alter the response of cells to an agent which calls forth such adaptive changes in an enzyme level, it is possible that more harm than good may result. Such compounds as actinomycin D or cycloheximide may actually enhance host toxicity by blocking adaptive changes in normal cells which make the primary agent tolerable without having a significant increase in the toxicity to malignant cells. These possibilities are amenable to experimental approach and would seem to be a profitable area for investigation. Hopefully, some converse logic may arise from further investigation on this point which will permit the design of better synergistic agents to selectively control the adaptive changes in tumor cells that may minimize the effectiveness of current therapy. This type of approach has been thoroughly reviewed by Sartorelli (19) under the term collateral sensitivity.

In the previous paper, Dr. Busch has presented concepts for the control of the malignant cell through administration of modified portions of the nuclear proteins and RNA. These are certainly attractive and worth the effort involved in their exploration. Another macromolecular assembly which should be appropriate for chemotherapeutic exploration is the cell membrane. The loss of contact inhibition in malignant transformation, and its possible association with the metastatic process, is under extensive investigation. Especially attractive at this time are the events of viral transformation of normal cells. It has been shown that the penetration of influenza virus into a host cell is associated with the cleavage of a sialic acid residue from the cell membrane by a neuraminidase activity in the virus (8). This process may be a part of the actual penetration step. Such a specialized and extracellular reaction would seem a very suitable target for antimetabolite activity. Although this appears more logical as a prophylactic measure to reduce initiation of the malignant process, it may also have relevance to continued reinfection of potential substrate cells if the reaction is found to be of importance with oncogenic viruses.

A related area of relevance to chemotherapy is the modification of the membrane of mammalian cells transformed by SV40 virus. Associated with viral infection is a reduction in the amount of sialic acid and N-acetyl hexosamine in the membrane. Further studies of enzymatically degraded membranes indicates distinctly different patterns in the labeling of glycopeptides from infected and normal cells (22). From these alterations in membrane structure it may be possible to derive a better understanding of the cellular interactions characteristic of malignant cells if in fact there is a common denominator in this area. From this could develop therapeutic measures which would attempt to minimize those changes which impart some of the undesirable properties of the malignant cell.

Finally, I would like to consider some specific ways in which the subunit structure of enzymes may be relevant to current interest in L-asparaginase (1). In the clinical use of this agent, two problems may be considered as limiting its effectiveness in the treatment of sensitive neoplasms. First, the enzyme has a molecular weight of 133,000 and, therefore, is confined largely to the vascular tree. A small amount of the enzyme has been shown to find its way into lymph (12) and presumably, therefore, into the interstitial space. Thus, the primary effect of therapy is to eliminate asparaginase from the plasma but only minimally from the immediate environment of many tumor cells interspersed in tissues. Undoubtedly many cells that required asparaginase for growth could derive this nutritional factor not from the plasma but from adjacent normal cells capable of producing and excreting this amino acid. One means of combating this cross feeding would be to devise analogs capable of inhibiting excess synthesis by normal cells. Some preliminary work in this area has been reported using asparagine (10) as well as glutamine (15) analogs since the latter amino acid is an alternative substrate with ammonia for the biosynthesis of asparagine in mammalian cells (17). A second problem encountered to variable degrees in different clinical groups has been the development of an immune reaction to this protein from E. coli. In some cases therapy has been terminated by near fatal anaphylactic reactions (4).

It is possible that some considerations of subunit structure now in progress could provide a reasonable solution to both of these problems. We have previously shown that the asparagine analog 5-diazo-7-oxo-L-norvaline (DONV) can interact both as a substrate and as an inactivator of the enzyme from E. coli, E.C. II (9). Recent studies by R. Jackson (14) in our laboratory have shown that it is possible to dissociate these actions by the organic solvent dimethylsulfoxide so that only alklation of the active site occurs. Using DONV-5,14C it can now be demonstrated that there are 4 binding sites on each molecule of 133,000 molecular weight. Tentatively, these data along with amino acid analysis and sedimentation studies by Whelan and Wriston (20) would suggest that this molecule is composed of subunits. If this is true, and the subunits are enzymatically active, it could be profitable to effect dissociation and modify the enzyme in some manner to prevent reassociation but retain enzymatic activity. Such subunits with a molecular weight of approximately 30,000 would be much more likely to penetrate into the interstitial space and perhaps thereby limit the cross feeding of L-asparaginase between normal cells and their neoplastic neighbors that require exogenous asparaginase. Such a reduction in molecular weight might also reduce significantly the antigenic potential of the material, since current evidence suggests that a reduction in molecular weight reduces the antigenic properties of proteins (21), and may even promote tolerance.

Although asparaginase may not offer therapeutic effectiveness greater than that available in other chemotherapeutic agents, it is effective in most acute lymphocytic leukemic patients who have become refractory to conventional treatment. More important it represents the first successful approach to enzyme therapy of cancer and may herald the appearance of other enzymes capable of creating selective nutritional or metabolic changes for the treatment of cancer and other metabolic disease.
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REFERENCES

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